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L1: Entry 1 of 1 File: USPT Jan 28, 2003

US-PAT-NO: 6511809
DOCUMENT-IDENTIFIER: US 6511809 B2

TITLE: Method for the detection of an analyte by means of a nucleic acid reporter

DATE-ISSUED: January 28, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
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APPL-NO: 09/ 858994 [PALM]
DATE FILED: May 16, 2001

PARENT-CASE:
This application claims the benefit of U.S. Provisional Application No. 60/211,293, filed Jun. 13, 2000.

INT-CL: [07] C12 Q 1/68, C12 P 19/34

US-CL-ISSUED: 435/6; 435/91.1, 435/91.2, 435/7.1
US-CL-CURRENT: 435/6; 435/7.1, 435/91.1, 435/91.2

FIELD-OF-SEARCH: 435/6, 435/91.1, 435/91.2, 435/7.1

PRIOR-ART-DISCLOSED:

U.S. PATENT DOCUMENTS

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	PAT-NO	ISSUE-DATE	PATENTEE-NAME	US-CL
☐	4668621	May 1987	Doellgast	
☐	4882269	November 1989	Schneider et al.	
☐	5424413	June 1995	Hogan et al.	536/24.31

☐	5648213	July 1997	Reddy et al.	435/6
☐	5665539	September 1997	Sano et al.	
☐	5985548	November 1999	Collier et al.	435/6
☐	6117631	September 2000	Nilsen	435/6

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FOREIGN-PAT-NO	PUBN-DATE	COUNTRY	US-CL
WO 93/15229	August 1993	WO	

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WO 89/03891, May 5, 1989, Chiron Corporation, Appl. No. PCT/US88/03644.
European Patent Application No. EO 204 510 A2. Filed May 29, 1986, Amoco Corporation.

ART-UNIT: 1637

PRIMARY-EXAMINER: Horlick; Kenneth R.

ABSTRACT:

A process is disclosed for the detection of an analyte utilizing a nucleic acid label as a reporter. The analyte is detected by the binding of at least two reporter reporter conjugates, each conjugate comprising a member of a binding pair and a nucleic acid label. The binding of the reporter conjugates to the analyte facilitates the juxtaposition of the nucleic acid labels, forming a single nucleic acid amplicon. The amplicon may then be detected directly, or may be used as a template of the generation of amplification products. Detection of the analyte by this process significantly reduces assay background caused by non-specific reporter conjugate binding.

26 Claims, 9 Drawing figures

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Perry; Michael P.	Downington	PA		

US-CL-CURRENT: 435/6; 435/7.1, 435/91.1, 435/91.2

CLAIMS:

What is claimed is:

1. A method for the detection of a non-nucleic acid analyte comprising: (i) contacting at least one non-nucleic acid analyte having at least two reporter conjugate binding sites with at least two reporter conjugates, said reporter conjugates each comprising: a) one member of a binding pair having specificity for at least one reporter conjugate binding site on said analyte; b) a nucleic acid label; wherein said analyte binds to said reporter conjugate forming an analyte dependent reporter complex; (ii) contacting said analyte dependent reporter complex with a enzyme composition wherein the nucleic acid labels on said reporter conjugates are joined to form an analyte specific amplicon; (iii) contacting the analyte dependent amplicon with an replication composition wherein amplification products are produced; and (iv) detecting said amplification products.

2. A method according to claim 1 wherein said non-nucleic acid analyte at step (i) is optionally immobilized on a solid support.

3. A method according to claim 1 wherein said enzyme composition comprises a DNA polymerase and wherein said nucleic acid labels on said reporter conjugates are joined by an overlap at each 3' end.

4. A method according to claim 1 wherein said enzyme composition comprises a DNA ligase and wherein said nucleic acid labels on said reporter conjugates are enzymatically joined by means of a ligation linker comprising a replication inhibitory moiety.

5. A method according to claim 3 wherein said overlap comprises from about 10 bases to about 30 bases.

6. A method according to claim 2 wherein said solid support is comprised of materials selected from the group consisting of polyethylene, polypropylene, poly(4-methylbutene), polystyrene, polymethacrylate, poly(ethylene terephthalate), nylon, poly(vinyl butyrate), glass, ceramics, metal and metal oxides.

7. A method according to claim 1 wherein said one member of a binding pair is selected from the group consisting of an antigen, antibody, hapten, nucleic acid, a nucleic acid aptamer, biotin, streptavidin, avidin, folic acid, folate binding protein, protein A protein G, immunoglobulins, epoxide, malaimide and sulfhydryl reactive groups.

8. A method according to claim 1 wherein the at least two reporter conjugates have different specificities for different reporter conjugate binding sites on said analyte.

9. A method according to claim 1 wherein said nucleic acid labels are of different lengths.

10. A method according to claim 1 wherein said nucleic acid labels are of different nucleotide sequence.

11. A method according to claim 1 wherein said nucleic acid labels are from about 30 bases to about 1000 bases in length.

12. A method for the detection of a non-nucleic acid analyte comprising: (i) immobilizing at least one non-nucleic acid analyte on a solid support, said analyte having at least two reporter conjugate binding sites; (ii) contacting said analyte with at least one reporter conjugate pair, said reporter conjugate pair comprising a first reporter conjugate and a second reporter conjugate, each of said first and second reporter conjugates further comprising: a) one member of a binding pair having an affinity for at least one reporter conjugate biding site on said analyte; b) a nucleic acid label; wherein said nucleic acid label of said first reporter conjugate comprises a 3' hydroxyl group and wherein said nucleic acid label of said second reporter conjugate comprises a 5' phosphoryl group and wherein said analyte binds to said reporter conjugate forming an analyte dependent reporter complex; (iii) contacting said analyte dependent reporter complex with a DNA ligase, wherein said first and second nucleic acid labels are ligated to form an analyte specific amplicon; (iv) contacting said analyte specific amplicon with a replication composition wherein said amplicon is amplified forming amplification products; and (v) detecting said amplification products.

13. A method according to claim 12 wherein at step (iii) a ligation linker comprising a 3' replication inhibitory moiety is optionally added together with said DNA ligase.

14. A method according to claim 13 wherein said replication inhibitory moiety is selected from the group consisting of dideoxynuleotides, a sequence of mismatched nucleotides, 3' phosphate and cordycepin.

15. A method according to claim 12 wherein said solid support is comprised of materials selected from the group consisting of polyethylene, polypropylene, poly(4-methylbutene), polystyrene, polymethacrylate, poly(ethylene terephthalate), nylon, poly(vinyl butyrate), glass, ceramics, metal and metal oxides.

16. A method according to claim 12 wherein said one member of a binding pair is selected from the group consisting of an antigen, antibody, hapten, nucleic acid, a nucleic acid aptamer, biotin, streptavidin, avidin, folic acid, folate binding protein, protein A protein G, immunoglobulins, epoxide, malaimide and sulfhydryl reactive groups.

17. A method according to claim 12 wherein the at least two reporter conjugates have different specificities for different reporter conjugate binding sites on said analyte.

18. A method according to claim 12 wherein said nucleic acid labels are of different lengths.

19. A method according to claim 12 wherein said nucleic acid labels are of different nucleotide sequence.

20. A method according to claim 12 wherein said nucleic acid labels are from about 25 bases to about 1000 bases in length.

21. A method for the detection of a non-nucleic acid analyte comprising: (i) contacting at least one non-nucleic acid analyte with at least one reporter conjugate pair, said reporter conjugate pair comprising a first reporter conjugate and a second reporter conjugate, each of said first and second reported conjugates further comprising: a) one member of a binding pair having an affinity for at least one reporter conjugate biding site on said analyte; b) a nucleic acid label; wherein said nucleic acid label of said first reporter conjugate comprises a 3' hydroxyl group and wherein said nucleic acid label of said second reporter conjugate comprises a 5' phosphoryl group and wherein said analyte binds to said reporter conjugate forming an analyte dependent reporter complex; (ii) contacting said analyte dependent reporter complex with a DNA ligase; wherein said first and second nucleic acid labels are ligated to form an analyte dependent amplicon; (iii) contacting said analyte specific amplicon with a replication composition wherein said amplicon is amplified forming amplification products; and (iv) detecting said amplification products.

22. A method for the detection of a non-nucleic acid analyte comprising: (i) contacting at least one non-nucleic acid analyte having at least two reporter conjugate binding sites with at least two reporter conjugates, said reporter conjugates each comprising: a) one member of a binding pair having specificity for at least one reporter conjugate binding site on said analyte; b) a nucleic acid label; wherein said analyte binds to said reporter conjugates forming an analyte dependent reporter complex; (ii) contacting said analyte dependent reporter complex with; a) an enzyme composition; and b) a nucleic acid reporting label selected from the group consisting of fluorescent moieties, chemiluminescent moieties, particles, enzymes, radioactive tags, light emitting moieties and intercalating dyes; wherein the nucleic acid labels on said reporter conjugates are joined to form an analyte specific amplicon and wherein said nucleic acid reporting label is incorporated into said amplicon; and (iii) detecting said labeled amplicon.

23. A method according to claim 22 wherein said enzyme composition comprises a DNA polymerase and wherein said nucleic acid labels on said reporter conjugates are joined by an overlap at each 3' end.

24. A method according to claim 22 wherein said enzyme composition comprises a DNA ligase and wherein said nucleic acid labels on said reporter conjugates are enzymatically joined by means of a ligation linker comprising a replication inhibitory moiety.

25. A method according to claim 22 wherein said non-nucleic acid analyte of step (i) is optionally immobilized on a solid support.

26. A method for the detection of a nucleic acid analyte comprising: (i) contacting at least one nucleic analyte having at least two reporter conjugate binding sites with at least two reporter conjugates, said reporter conjugates each comprising: a) one member of a binding pair having specificity for at least one reporter conjugate binding site on said analyte, the one member of a binding pair selected from the group consisting of an antigen, antibody, biotin, streptavidin, avidin, folic acid, folate binding protein, protein A protein G, immunolobulins, epoxide, malaimide and sulfhydryl reactive groups; b) a nucleic acid label; wherein said analyte binds to said reporter conjugates forming an analyte dependent reporter complex; (ii) contacting said analyte dependent reporter complex with a enzyme composition wherein the nucleic acid labels on said reporter conjugates are joined to form an analyte specific amplicon; (iii) contacting the analyte specific amplicon with an replication composition wherein amplification products are produced; and (iv) detecting said amplification products.

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☐ 1. 6511809. 16 May 01; 28 Jan 03. Method for the detection of an analyte by means of a nucleic acid reporter. Baez; Luis, et al. 435/6; 435/7.1 435/91.1 435/91.2. C12Q001/68 C12P019/34.

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